REMARKS

As will be discussed below, claims 1, 3 and 4 have been amended to more distinctly claim that which Applicants regard as their invention. Specifically claim 1 has been amended to recite that an increased matrix metalloproteinase-9 activity indicates increased probability of establishing pregnancy and to address the 35 U.S.C. §112, second paragraph rejections. Claims 3 and 4 have been amended to address the 35 USC §112 rejections.

1. **The Rejections Under 35 U.S.C. 112, Second Paragraph**

Claims 1-4 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, it is stated in the Office Action:

> On lines 2-3 of claim 1, the phrase "the follicular fluid" lacks antecedent basis. On line 3 of claim 1, it is suggested to insert the phrase - collected from the human female – after the word "oocyte".
>
> On line 2 of claim 3, it is suggested to change the phrase "wherein the diameter of the follicles selected is not less than 17mm" to – wherein the follicular fluid is collected from a follicle having a diameter not less than 17 mm – since claim 1 does not positively recite the follicles.
>
> On line 2 of claim 4, the phrase "said follicle" lacks antecedent basis.

In response, claim 1 has been amended to be directed to method for predicting pregnancy outcome in a human female subject comprising measuring the activity of matrix metalloproteinase-9 in follicular fluid of a matured oocyte from a human female. Claim 3 has been amended to recite that the follicular fluid is collected from a follicle having a diameter hot less than 17 mm. Claim 4 has been amended to recite that the method further comprises obtaining said follicular fluid from a follicle of said mature oocyte.

In view of the amendments of claims 1, 3 and 4 and the above arguments, Applicants assert that the rejections under 35 U.S.C. §112, second paragraph have been overcome. Therefore, Applicants respectfully request that the rejections be withdrawn.

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2. The Rejections Under 35 U.S.C. §103

Claims 1-5 have been rejected over Shalev et al. Claims 8-9 are rejected under 35 U.S.C. 103 (a) as being unpatentable over Shalev et al in view of the Molecular Probes brochure on the Uncheck Gelatinase/Collegenase assay kit (cited in the Office action mailed on July 29, 2004). These rejections are discussed in detail below.

2.1 The Rejection of Claims 1-5 (Shalev et al.)

The Office Action specifically states:

> Shalev et al. teach of a method for measuring different matrix metalloproteinases, including matrix metalloproteinase-9 (MMP-9), in the follicular fluid of women undergoing induction of ovulation for in vitro fertilization. In the method, follicular fluid samples retrieved from the follicles of mature oocytes are collected from both women who undergo normal ovulation and women affected by polycystic ovarian syndrome (PCOS). Women who have PCOS are characterized by a degree of infertility. The follicular fluid samples are collected from follicles at least 18 mm in diameter. The level of MMP-9 in the follicular fluid samples is measured by substrate gel electrophoresis or zymography where the fluid samples are applied to a gel-containing gelatin as the substrate for MMP-9. Any MMP-9 in the samples serves to digest the gelatin in the gel. See page 326 in Shalev et al. Shalev et al. teach that the level of MMP-9 in the follicular fluid of the women having PCOS is greater than in the women who undergo normal ovulation. See the results section of page 327 of Shalev et al. Shalev et al. teach that it is known in the art that MMP-9 is present in the ovaries of humans, rats and mice, and this MMP allows the development of ovarian follicles, the breakdown of the follicular wall to release a mature oocyte at the time of ovulation, and the formation of the corpus lustrum from leutinizing follicular cells. See page 329 of Shalev et al. Shalev et al. also teach that the high gelatinolytic activity by MMP-9 in the PCOS women could contribute to the rapid regression of the corpus luteum and consequently lead to insufficient luteal function for pregnancy to occur. See the first column of page 330 of Shalev et al.
>
> Shalev et al. fail to teach that the method for measuring MMP-9 in follicular fluid samples can be used to determine the probability of establishing pregnancy in the human female. However, it would have been obvious to one of ordinary skill in the art at the time of

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> the instant invention to use the method taught by Shalev et al for such a purpose since Shalev et al. teach that MMP-9 levels in fertile women differ from the levels found in infertile women, and also teach that MMP-9 contributes to the release of a mature oocyte from a follicle during ovulation, and in order for successful fertilization to occur, an oocyte must be released from a follicle for interaction with a sperm cell.

Applicants respectfully traverse the rejection. Before, responding to the rejection, Applicants would like to summarize the subject matter of the invention. Specifically, the present invention relates to the method of predicting pregnancy outcome in a patient by measuring the activity of matrix-metalloproteinase-9 enzyme (MMP-9) in the follicular fluid. As shown in the instant application, increased levels of matrix metalloproteinase-9 in the follicular fluid was found to be correlated with increased likelihood of a successful pregnancy. Surprisingly, no such correlation was found with another matrix metalloproteinase, matrix metalloproteinase 2. High levels of matrix metalloproteinase 2 were detected in the follicular fluid of both women who did and did not ultimately become pregnant.

With respect to the cited reference, Applicants note that Shalev et al found that patients with polycystic ovarian syndrome (PCOS) had higher levels of MMP-9 than normal ovulatory women. The present invention certainly has no relationship with any special condition of disease such as PCOS. The observation of Shalev et al. certainly has little relevance to the subject matter of the present invention, use of MMP-9 levels in ART patients' follicular fluid as a predictor of successfully pregnancy outcome. Actually, the teaching of Shalev et al. that MMP-9 and MMP-2 levels are increased in women with PCOS may actually indicate that MMP-9 and MMP-2 impede pregnancy. Applicants wish to emphasize that there was no suggestion in Shalev et al. indicating any direct relationship between MMP-9 level and the pregnancy outcome.

A number of investigators verified that the change of extracellular matrix was accompanied during the implanting process and MMP molecules participated in the ovulation and the implantation process thereof (see, for example, Hulboy et al, 1997, Mol. Hum. Reprod. 3:27-45, submitted in the IDS). However, the results of previous studies could not confirm any direct relationships between each level of extracellular matrix or matrix metallproteinases and

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the pregnancy outcome level. At best, these studies only suggest that metalloproteinases play a role in follicular development. For example, it is stated in Bagavandoss, 1998, J. Endocrinol. 158:221-228, submitted as a Supplemental Information Disclosure Statement) on page 227:

> In summary, the results of this study indicate that the gelatinases MMP-2 and MMP-9, and TIMP-1 are differentially distributed in the neonatal and gonadotropin-primed rat ovaries before ovulation and during pseudopregnancy. The unique spatial and temporal expression of these proteases and their inhibitor suggests a diverse role for these proteins in follicular development, ovulation and the development, maintenance and regression of the corpus luteum.

Actually, Shalev et al. states

> Three MMP have been detected in human luteinized granulose cells. These are MMP-1, MMP-2 and MMP-9 (citations omitted). Although several intraovarian regulators, such as IL-1 have been found to influence the level of expression of these respective MMP, it is not fully understood what may be the pathophysiological significance of their altered expression (citation omitted).

Applicants also note that in of of the references cited by the Examiner, Lahav-Baratz, the activities of MMP-2 and MMP-9 were similar in women with PCOS and normal ovulating women. Explanations were offered on page 570, col. 2 of Lahav-Baratz. However, given the contradictory teachings of Shalev and Lahav-Baratz, one of orndinary skill in the art would not have concluded that the level of MMP-9 in the follicular fluid of the women having PCOS is greater than in the women who undergo normal ovulation.

One of ordinary skill in the art would not be provided with any direction as to the significance of alteration of MMP-1, MMP-2 or MMP-9 levels and whether MMP-1, MMP-2 or MMP-9 levels could be correlated to pregnancy outcome. Thus one of ordinary skill in the art would certainly not have a reasonable expectation of success that MMP-9 levels would be an accurate predictor of pregnancy outcome.

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In view of the above arguments, it is Applicants position that the claims 1-5 are not obvious over Shalev et al. Therefore, Applicants respectfully request that the rejection be withdrawn.

2.2 The Rejection of Claims 8-9

Claims 8-9 are rejected under 35 U.S.C. 103 (a) as being unpatentable over Shalev et al in view of the Molecular Probes brochure on the EnzChek Gelatinase/Collegenase assay kit (cited in the Office action mailed on July 29, 2004). It is asserted that the Enz/Chek Gelatinase/Collagenase Assay kit by Molecular Probes is used to measure the gelatinase or collegenase activity of matrix metalloproteinases (MMPs). The kit contains a component a protein substrate, which can be digested by an MMP. The protein substrate is gelatin, collagen I or collagen IV. See pages 1-2 of the brochure. It is concluded in the Office Action that

> Based upon the combination of Shalev et al and the brochure of the EnzChek Gelatinase/Collagenase Assay kit by Molecular Probes, it would have been obvious to one of ordinary skill in the art to utilize the kit taught by the brochure for performing the method taught by Shalev et al since the method taught by Shalev et al involves zymography with a gelatin electrophoresis gel in order to measure the gelatinase activity of MMPs, and the kit in the brochure is also used to measure the gelatinase activity of the MMPs. The kit taught by the brochure would allow the quick and efficient performance of the method taught be Shalev et al by having all of the reagents and other components needed to perform the method present in one place in the proper concentrations.

Applicants respectfully traverse the rejection. As noted above, Shalev et al. compares MMP-9 and MMP-2 levels in normal females and patients with PCOS. At best, it teaches that MMP-9 and MMP-2 levels are increased in PCOS patients. However, as noted above, a later reference cited by the examiner, Lahav-Baratz, teaches that there was no alteration in MMP-9 and MMP-2 levels in PCOS patients and normal ovulating women. There is no suggestion that increased MMP-9 levels can be correlated with increased probability of achieving a successful pregnancy outcome. Therefore, claims 8 and 9 would not be obvious in view of Shalev et al.

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The secondary reference, the Molecular Probes Brochure would not add anything of significance to the disclosure of Shalev et al. It is merely an information sheet detailing procedure used for assaying MMP activity. There is no suggestion in this reference as to specific circumstances when the assay may be used. It is only a protocol sheet. Given that the primary reference, Shalev et al. only teaches that MMP-9 and MMP-2 levels increase in patients with PCOS and that MMP-9, MMP-1 and MMP-2 are present throughout the ovulatory cycle but no teaching regarding significance of the alteration of levels of these metalloproteinases, it would not be obvious to combine this reference with a reference merely detailing the MMP assay method to obtain the method of the present invention, a method for predicting pregnancy outcome.

In view of the above arguments, it is Applicants position that the claims 8-9 are not obvious over Shalev et al. in view of the Molecular Probes brochure are not obvious. Therefore, Applicants respectfully request that the rejection be withdrawn.

3.Conclusion

In view of the foregoing, Applicants assert that the claims are now in condition for allowance. Early action to that end is respectfully requested. The Examiner is invited to contact the undersigned at (914) 712-0093 if she has any questions.

Respectfully submitted,

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